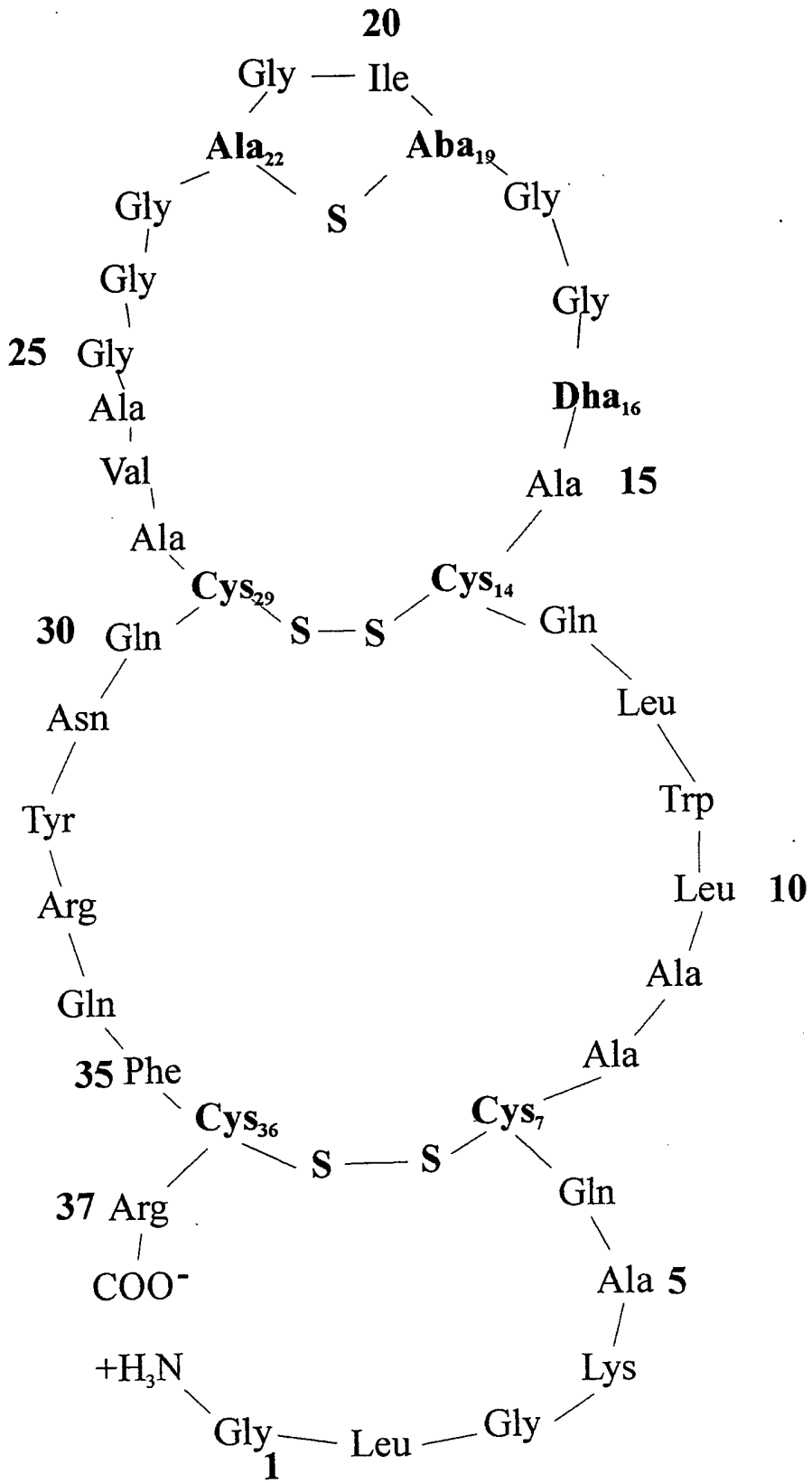


Sublancin 168 **Figure 1**

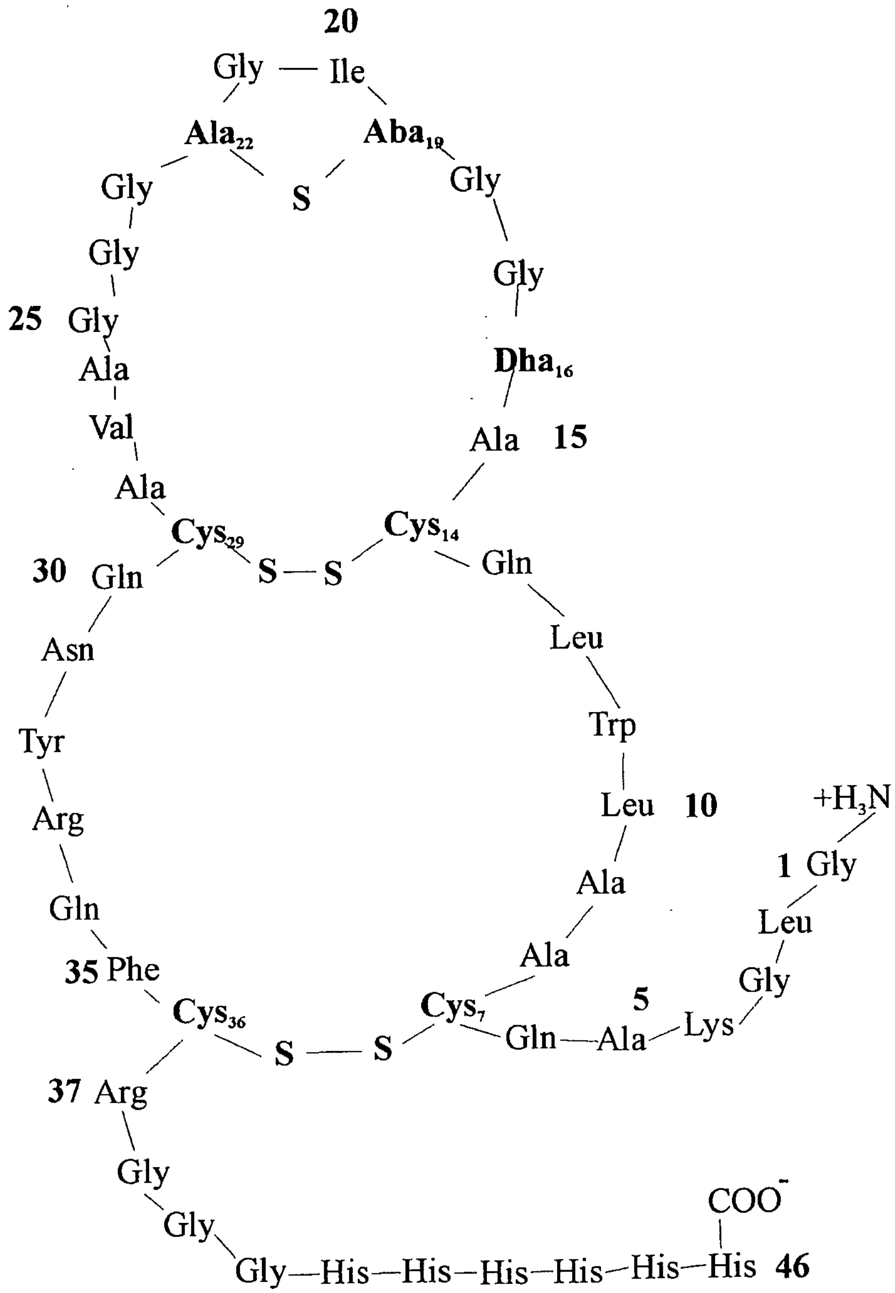
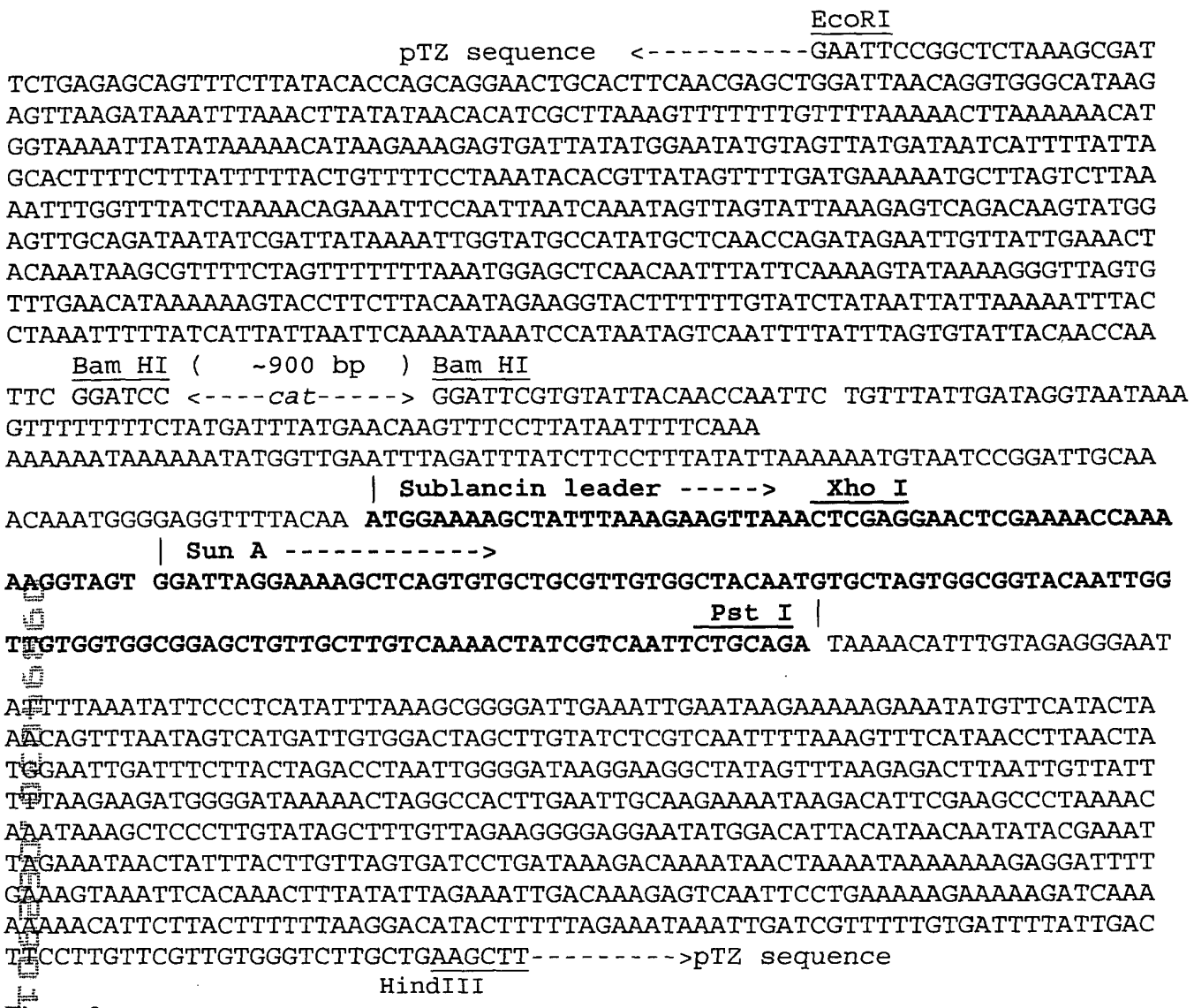


Figure 2

Sublancin-His tag


EcoRI
pTZ sequence <----------GAATTCCGGCTCTAAAGCGAT
TCTGAGAGCAGTTTCTTATACACCAGCAGGAACTGCACTTCAACGAGCTGGATTAACAGGTGGGCATAAG
AGTTAAGATAAATTTAAACTTATATAACACATCGCTTAAAGTTTTTTTGTTTTAAAAACTTAAAAAACAT
GGTAAAATTATATAAAAACATAAGAAAGAGTGATTATATGGAATATGTAGTTATGATAATCATTTTATTA
GCACTTTTCTTTATTTTTACTGTTTTCCTAAATACACGTTATAGTTTTGATGAAAAATGCTTAGTCTTAA
AATTTGGTTTATCTAAAACAGAAATTCCAATTAATCAAATAGTTAGTATTAAAGAGTCAGACAAGTATGG
AGTTGCAGATAATATCGATTATAAAATTGGTATGCCATATGCTCAACCAGATAGAATTGTTATTGAAACT
ACAAATAAGCGTTTTCTAGTTTTTTTAAATGGAGCTCAACAATTTATTCAAAAGTATAAAAGGGTTAGTG
TTTGAACATAAAAAAGTACCTTCTTACAATAGAAGGTACTTTTTTGTATCTATAATTATTAAAAATTTAC
CTAAATTTTTATCATTATTAATTCAAAATAAATCCATAATAGTCAATTTTATTTAGTGTATTACAACCAA
Bam HI (~900 bp) Bam HI
TTC GGATCC <----cat-----> GGATTCGTGTATTACAACCAATTC TGTTTATTGATAGGTAATAAA
GTTTTTTTTCTATGATTTATGAACAAGTTTCCTTATAATTTTCAAA
AAAAAATAAAAAATATGGTTGAATTTAGATTTATCTTCCTTTATATTAAAAAATGTAATCCGGATTGCAA
| Sublancin leader -----> Xho I
ACAAATGGGGAGGTTTTACAA ATGGAAAAGCTATTTAAAGAAGTTAAACTCGAGGAACTCGAAAACCAAA
| Sun A ------------>
AAGGTAGT GGATTAGGAAAAGCTCAGTGTGCTGCGTTGTGGCTACAATGTGCTAGTGGCGGTACAATTGG
Pst I |
TTGTGGTGGCGGAGCTGTTGCTTGTCAAAACTATCGTCAATTCTGCAGA TAAAACATTTGTAGAGGGAAT
ATTTTAAATATTCCCTCATATTTAAAGCGGGGATTGAAATTGAATAAGAAAAAGAAATATGTTCATACTA
AACAGTTTAATAGTCATGATTGTGGACTAGCTTGTATCTCGTCAATTTTAAAGTTTCATAACCTTAACTA
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AAATAAAGCTCCCTTGTATAGCTTTGTTAGAAGGGGAGGAATATGGACATTACATAACAATATACGAAAT
TAGAAATAACTATTTACTTGTTAGTGATCCTGATAAAGACAAAATAACTAAAATAAAAAAAGAGGATTTT
GAAAGTAAATTCACAAACTTTATATTAGAAATTGACAAAGAGTCAATTCCTGAAAAAGAAAAAGATCAAA
AAAAACATTCTTACTTTTTTAAGGACATACTTTTTAGAAATAAATTGATCGTTTTTGTGATTTTATTGAC
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HindIII

Figure 3

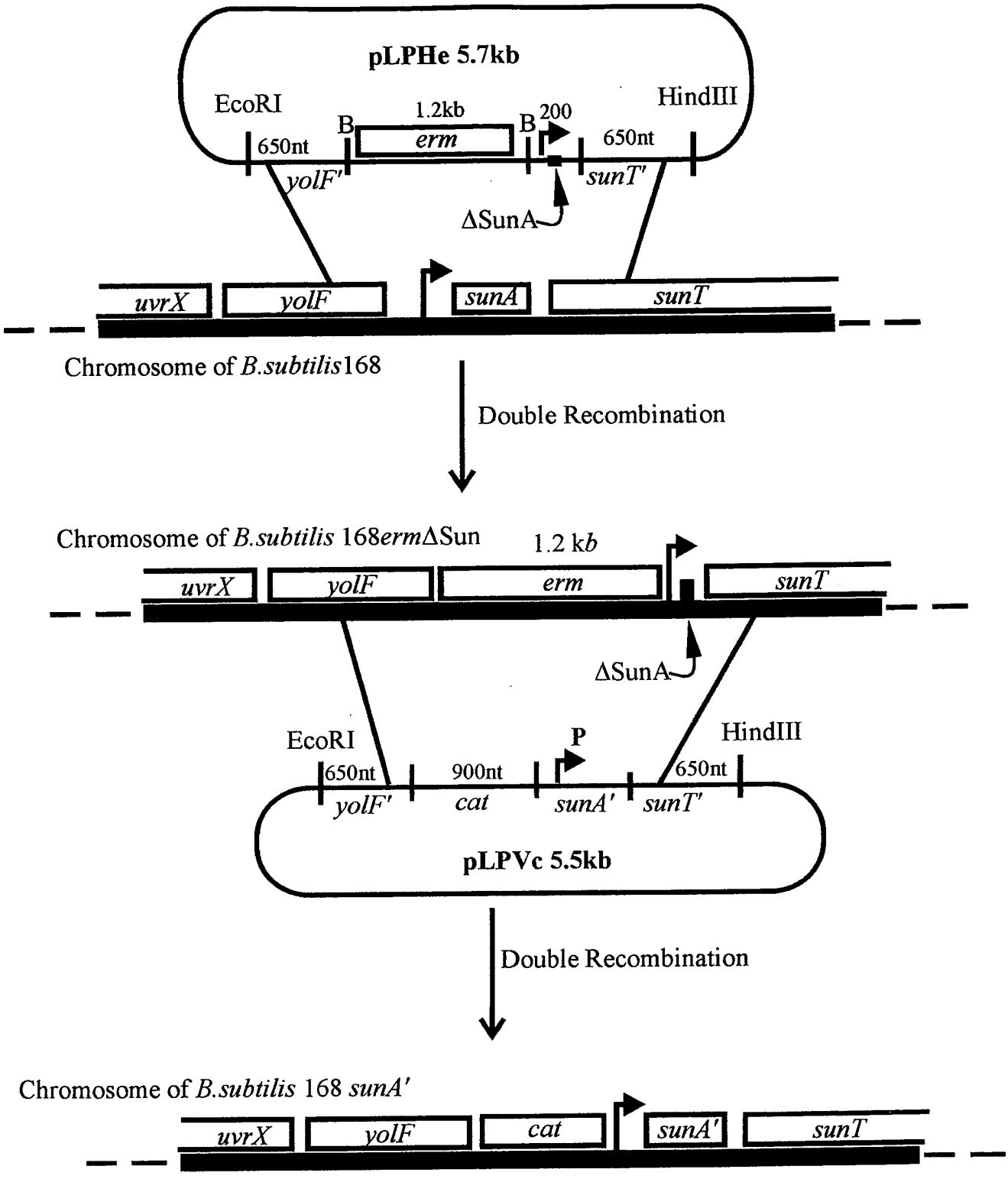
pLPHe 5.7kb
EcoRI
1.2kb
B
B
200
HindIII
650nt
erm
650nt
yolF'
sunT'
ΔSunA
uvrX
yolF
sunA
sunT
Chromosome of B.subtilis168
Double Recombination
Chromosome of B.subtilis 168ermΔSun
1.2 kb
uvrX
yolF
erm
sunT
ΔSunA
EcoRI
P
HindIII
650nt
900nt
650nt
yolF'
cat
sunA'
sunT'
pLPVc 5.5kb
Double Recombination
Chromosome of B.subtilis 168 sunA'
uvrX
yolF
cat
sunA'
sunT

Figure 4

Figure 5

```
←—— pLPcat
                                  Sublancin leader→
TTGCAAACAAATGGGGAGGTTTTACAA ATGGAAAAGCTATTTAAAGAAG
                            MetGluLysleuPheLysGluV

     XhoI                              sublancin prep-
TTAAACTCGAGGAACTCGAAAACCAAAAAGGTAGT GGATTAGGAAAAGC
AlLysLeuGluGluLeuGluAsnGluLysGlySer GlyLeuGlyLysAl

tide→
TCAGTGTGCTGCGTTGTGGCTACAATGTGCTAGTGGCGGTACAATTGGTT
aGlnCysAlaAlaLeuTrpLeuGlnCysAlaSerGlyGlyThrIleGlyC

       KasI
GTGGTGGCGGCGCCGTTGCTTGTCAAAACTATCGTCAATTCTGTAGAGGT
ysGlyGlyGlyAlaValAlaCysGlnAsnTyrArgGlnPheCysArgGly

     His Tag→           Stop    PstI
GGTGGTCATCATCATCATCATCATTAGAGTCCTGCAGATAAAACA
GlyGlyHisHisHisHisHisHis *     pLPcat ——→
```

Figure 6